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Cyr61 promotes breast tumorigenesis and cancer progression

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Cyr61, a member of the CCN family of genes, is an angiogenic factor. We have shown that it is overexpressed in invasive and metastatic human breast cancer cells and tissues. Here, we investigated whether Cyr61 is necessary and/or sufficient to bypass the 'normal' estrogen (E2) requirements for breast cancer cell growth. Our results demonstrate that Cyr61 is sufficient to induce MCF-7 cells to grow in the absence of E2. Cyr61-transfected MCF-7 cells (MCF-7/Cyr61) became E2-independent but still E2-responsive. On the other hand, MCF-7 cells transfected with the vector DNA (MCF-7/V) remain E2-dependent. MCF-7/Cyr61 cells acquire an antiestrogen-resistant phenotype, one of the most common clinical occurrences during breast cancer progression. MCF-7/Cyr61 cells are anchorage-independent and capable of forming Matrigel outgrowth patterns in the absence of E2. ERα expression in MCF-7/Cyr61 cells is decreased although still functional. Moreover, MCF-7/Cyr61 cells are tumorigenic in ovariectomized athymic nude mice. The tumors resemble human invasive carcinomas with increased vascularization and overexpression of vascular endothelial growth factor (VEGF). Our results demonstrate that Cyr61 is a tumor-promoting factor and a key regulator of breast cancer progression. This study provides evidence that Cyr61 is sufficient to induce E2-independence and antiestrogen-resistance, and to promote invasiveness *in vitro*, and to induce tumorigenesis *in vivo*, all of which are characteristics of an aggressive breast cancer phenotype.
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About 60% of human breast carcinomas expressing estrogen receptor (ER), are dependent upon estrogen (E2) for growth, and thus respond to treatment with an ER antagonist, tamoxifen (Tam). Many breast carcinomas, however, become less sensitive over time to estrogen, and thus more resistant to the endocrine treatment, developing into more aggressive tumors. Aggressiveness of breast cancer cells is commonly attributed to the ability of the cells to overcome E2 requirements for growth, and in most cases to acquire antiestrogen-resistance. However, the mechanism by which breast cancer progresses from an E2-dependent and antiestrogen-responsive phenotype to an E2-independent and antiestrogen-resistant phenotype has not yet been determined.

We have shown previously that expression of heregulin (HRG), a growth factor that activates the *erbB*-2/3/4 receptor signaling pathways, is closely associated with an invasive breast cancer phenotype (Cardillo *et al*., 1995). We have further demonstrated that HRG induces breast cancer progression, as determined by the loss of ER function and E2 response, tumorigenicity, invasion, and metastasis (Tang *et al*., 1996; Lupu *et al*., 1995, 1996; manuscript submitted for publication). We have hypothesized that HRG induces activation of the *erbB* signaling pathways, leading to regulation of downstream genes that control cancer cell growth and tumor progression. We thus isolated and identified Cyr61, an angiogenic factor, which is differentially expressed in ER-negative, HRG-positive, invasive, and metastatic breast cancer cells, and in 30% of breast tumor biopsies (Tsai *et al*., 2000). We showed that Cyr61 is important for HRG-mediated chemomigration and invasiveness of breast cancer cells *in vitro* (Tsai *et al*., 2000).

Cyr61 belongs to the CCN family of angiogenic regulators, which consists of Cyr61, CTFG, Nov, WISP-1, WISP-2, and WISP-3 (Lau and Lam, 1999). Cyr61 is a cysteine-rich, heparin-binding protein that is secreted and associated with the cell surface and the extracellular matrix (ECM) (Yang and Lau, 1991; Kireeva *et al*., 1997). It has been shown that Cyr61 binds to integrins, such as αvβ3, αIIbβ3 and α6β1 (Kireeva *et al*., 1996a; Jedsadayanmata *et al*., 1999; Chen *et al*., 2000). Cyr61 mediates cell adhesion, stimulates cellular migration, enhances growth factor-induced DNA synthesis in fibroblasts and endothelial cells, and increases chondrogenesis in mesenchymal cells (O'Brien and Lau, 1992; Kireeva *et al*., 1996b,

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1997; Frazier *et al*., 1996; Kolesnikova and Lau, 1998). Moreover, Cyr61 stimulates an integrin $\alpha v\beta 3$-dependent chemotaxis of endothelial cells (Babic *et al*., 1998). Most significantly, expression of Cyr61 enhances neovascularization and tumor formation of human tumor cells in immunodeficient mice (Babic *et al*., 1998, 1999; Xie *et al*., 2001).

To determine whether expression of Cyr61 is necessary and/or sufficient to promote breast cancer progression, Cyr61 was introduced into the MCF-7 breast cancer cells, which are ER-positive, E2-responsive *in vitro*, E2-dependent *in vivo*, and never metastasize *in vivo*. MCF-7 cells were stably transfected by electroporation with a eukaryotic expression vector, pcDNA3.1/zeocine(−) (Invitrogen), containing the full-length cDNA of the human Cyr61 gene (MCF-7/Cyr61), or with an empty vector (MCF-7/V) as a negative control. A number of Cyr61- (20 clones) and vector-transfected clones (10 clones) were isolated and characterized for the expression of Cyr61 at both the mRNA and protein levels. Cyr61 was highly expressed in the Cyr61-transfected cells as determined by the RNase protection assay (Figure 1a) and by Western blot analysis using conditioned media concentrated from the Cyr61 clones or vector control clones (Figure 1b). Importantly, the expression level of Cyr61 in MCF-7/Cyr61 was comparable to that in MDA-MB-231, which is an aggressive breast cancer cell line and naturally expresses high levels of Cyr61. Our results demonstrated a 10- to 35-fold increase in Cyr61 mRNA and/or protein in the MCF-7/Cyr61 cells, as compared with the wild type or MCF-7/V cells in which Cyr61 expression was very low or nearly undetectable (Figure 1). Since Cyr61 mRNA and protein expression did not vary significantly, and their cellular behavior was similar in most of the clones, we chose to present the data obtained in one vector clone (V2) and two representative Cyr61 clones (C6 and C20).

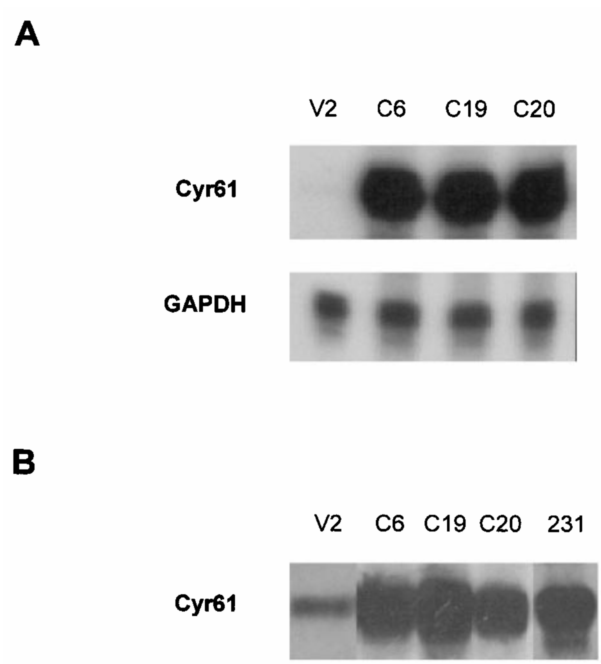

Figure 1 Expression of Cyr61 in MCF-7/Cyr61 clones. (**a**) MCF-7 cells were stably transfected by electroporation with a eukaryotic expression vector, pcDNA3.1/zeocine(−) (Invitrogen), containing the full-length cDNA of the human Cyr61 gene, or with an empty vector as a negative control. Transfected MCF-7 cells were selected in the presence of antibiotic zeocine (200 μg/ml) for 2 weeks. Individual vector transfectants (MCF-7/V, 10 clones) and Cyr61 transfectants (MCF-7/Cyr61, 20 clones) were isolated and grown. Total RNA was isolated from subconfluent MCF-7/V2 and MCF-7/Cyr61 cells, and 30 μg of RNA was analysed for Cyr61 mRNA expression by RNAse protection assay as previously described (Tsai *et al*., 2000). The GAPDH probe was used as an internal control for RNA loading. Representative vector (V2) and Cyr61 clones (C6, C19, and C20) were shown. (**b**) Subconfluent breast cancer cell lines were cultured in serum-free media for 48 h. Conditioned media were collected, concentrated 100×, and analysed by Western blotting with a rabbit polyclonal anti-Cyr61 antibody as previously described (Tsai *et al*., 2000)

The MCF-7/Cyr61 cells showed a growth advantage in E2-depleted media, having a 3–5-fold increase in growth as compared with the MCF-7/V cells (Figure 2a). The average doubling time in E2-free conditions for the MCF-7/Cyr61 cells is approximately 36 h, in contrast with 72 h for the MCF-7/V2 cells (data not shown). These results demonstrate that MCF-7/Cyr61 cells acquire an E2-independent phenotype. We thus postulate that overexpression of Cyr61 provides MCF-7 cells a growth advantage to bypass their ‘normal’ estrogenic requirement for cellular proliferation. As expected, E2 stimulated the growth of MCF-7/V2 cells (Figure 2a) because MCF-7 cells are E2-responsive (Pratt and Pollak, 1993). Although the MCF-7/Cyr61 cells acquire E2-independence, these cells are still responsive to E2 (Figure 2a), resembling one of the clinical phenotypes found in women suffering from breast cancer. We next tested the ability of antiestrogens to block the E2 induction of cell growth. Two distinct antiestrogens were used, Tamoxifen (Tam) and ICI 182,780 (ICI). Tam, a well-known antiestrogen, functions as an agonist and antagonist through both transcriptional activation domains (AF1 and AF2) of ER. ICI, a pure antiestrogen, acts solely as an antagonist through the AF1 domain (MacGregor and Jordan, 1998). When MCF-7/Cyr61 cells were treated with ICI (Figure 2a), and Tam (data not shown), both antiestrogens reduced only the growth induced by E2 in MCF-7/Cyr61 cells. They were unable to block the E2-independent growth of the MCF-7/Cyr61 cells, indicating that Cyr61 provides a true growth advantage that is not inhibited by antiestrogens. In other words, both antiestrogens inhibited the growth of the cells only to the basal level elevated by overexpression of Cyr61; they were not able to reduce cell growth to the same level observed in the wild type or MCF-7/V2 cells in the absence of E2 (Figure 2a). The fact that both antiestrogens had similar effects supports the hypothesis that the growth stimulation by E2 is indeed mediated directly through ER and most probably not mediated through other secondary mechanisms.

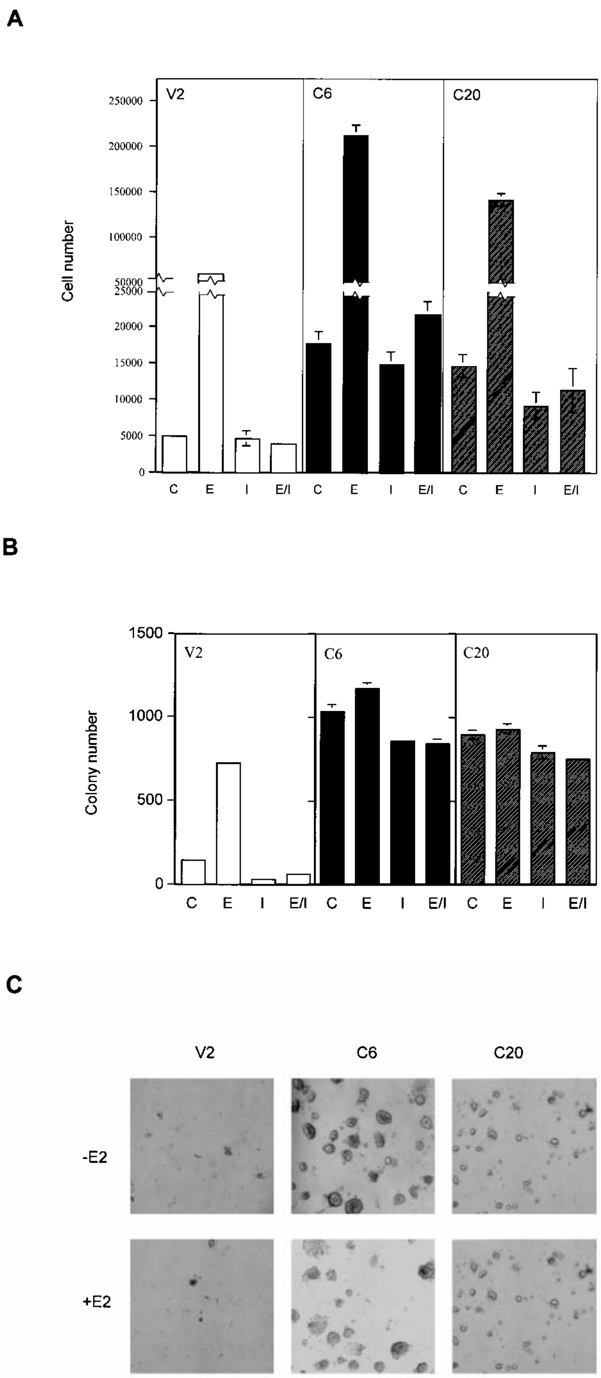

Figure 2 Induction of E2-independence and antiestrogen resistance of MCF-7 cells by Cyr61. (**a**) Subconfluent MCF-7/V2 and MCF-7/Cyr61 clones were cultured in media containing charcoal stripped fetal bovine serum (FBS) for 4 days and plated (2500 cells/well) in triplicate in 24-well plates for proliferation assays. Cells were incubated in the presence of solvent control (0.1% ethanol; C), E2 (1 nM; E), ICI (100 nM; I), and the combination of E2 and ICI (E/I). Cell number was counted at day 7. Data of two representative Cyr61 clones are shown from at least four independent experiments. Similar results were obtained from other Cyr61-overexpressing clones. (**b**) Subconfluent MCF-7/V2 and MCF-7/Cyr61 clones were cultured in E2-depleted media for 4 days and plated (20 000 cells/well) in triplicate in 6-well plates for anchorage-independent soft agar assay as previously described (Guerra-Vladusic *et al*., 1999). Cells were cultured in the presence of solvent control (C), E2 (E), ICI (I), and the combination of E2 and ICI (E/I) as described above for **a**. Data of two representative clones are shown from at least four independent experiments. Similar results were obtained from other Cyr61-overexpressing clones. (**c**) Subconfluent MCF-7/V2 and MCF-7/Cyr61 clones were cultured in E2-depleted media for 4 days and plated (5000 cells/well) in triplicate in 12-well plates in the presence or absence of E2 in the Matrigel Outgrowth assay as previously described (Hijazi *et al*., 2000). The outgrowth pattern was developed and photographed after a 10-day incubation. Two representative clones are shown with similar results from three independent experiments. Similar results were obtained from other Cyr61-overexpressing clones

To sum up, our data demonstrate that overexpression of Cyr61 stimulates cell growth of E2-dependent cells in the absence of E2, resulting in cells becoming E2-independent. On the other hand, E2 further enhanced cell proliferation of Cyr61-expressing cells, demonstrating that these cells, although independent of E2, are still responsive to E2. Consistent with our previous findings that E2 induces Cyr61 expression, and Tam and ICI blocks E2-induced Cyr61 expression (Tsai *et al*., 2002), here we demonstrated that both antiestrogens decrease E2-stimulated growth in Cyr61-expressing cells only to those levels already stimulated by overexpression of Cyr61. Therefore, it is overexpression of Cyr61 alone, which most probably accounts for the growth advantage observed in MCF-7 cells in E2-depleted conditions. Moreover, our *in vitro* studies represent a situation similar to what often occurs in human breast carcinomas *in vivo*, in which E2 can induce tumor growth (or not), in spite of levels of ER expression which are almost identical. Clinical trials have been conducted with E2 followed by chemotherapeutic drugs, based on the rationale that E2 stimulates tumor growth, allowing cells to enter the cell cycle, and thus provides a better environment for chemotherapeutic drugs to be more effective.

Cyr61-induced E2-independence became more evident when we further demonstrated that MCF-7/Cyr61 cells are anchorage-independent in the absence of E2 (Figure 2b), that is, these cells form colonies in soft agar assays. It is well established that MCF-7 cells are not anchorage-independent in the absence of E2. Colonies observed, if any, represent the background level for the colony formation assay. In general, the MCF-7/Cyr61 cells formed large colonies in the size range of 100 – 150 μm. E2 slightly stimulated the colony formation in the MCF-7/Cyr61 cells (Figure 2b). ICI was not able to block the colony formation induced by E2 in MCF-7/Cyr61 (Figure 2b). All other MCF-7/Cyr61 clones behaved similarly (data not shown). Neither vector nor wild type MCF-7 cells formed significant numbers of colonies in the absence of E2. Smaller colonies or single cells were seen in MCF-7/V2 cells in the presence of E2 (Figure 2b). As expected, E2 induced anchorage-independent growth of MCF-7/V cells, which was completely blocked by antiestrogens ICI (Figure 2b) and Tam (data not shown).

To determine whether Cyr61 promotes an invasive phenotype, MCF-7/Cyr61 cells were tested in the Matrigel outgrowth assay, which is frequently employed as a reliable system to assess *in vitro* invasiveness of breast cancer cells (Sommers *et al*., 1994; Hijazi *et al*., 2000). The MCF-7/Cyr61 clones C6 and C20 showed extensive outgrowth in Matrigel, the colonies appearing large and irregular in shape. In contrast, the MCF-7/V2 cells were not able to migrate through and proliferate in the Matrigel matrix, remaining as single cells in the matrix even in the presence of E2 (Figure 2c). Significantly, Cyr61 promotes outgrowth of MCF-7 cells in the Matrigel matrix in the absence of E2, suggesting that Cyr61 is capable of inducing a critical invasive phenotype of breast cancer cells in an E2-independent manner. These results strongly indicate that expression of Cyr61 enhances the *in vitro* invasiveness of breast cancer cells, which may thereby provide the appropriate milieu for these cells to migrate and perhaps metastasize *in vivo*. All other MCF-7/Cyr61 clones behaved similarly (data not shown).

It has been shown previously that a possible mechanism to acquire an E2-independent and anti-estrogen-resistant phenotype acts via the loss of ER expression and/or ER function. The ER expression was determined by RNase Protection assays; the ER function was determined as the ability of E2 to regulate the expression of E2-responsive genes (Govind and Thampan, 2001). We first examined the basal level of ER expression in MCF-7/Cyr61. We established only the expression of ERα, not ERβ, because the level of ERβ expression is extremely low in MCF-7 cells, and the role of ERβ in breast cancer is still unclear (Vladusic *et al*., 2000). The basal level of ERα expression is defined as ERα expression in the absence of E2 in cells cultured in phenol red-free media containing 5% charcoal-treated fetal bovine serum. Our data indicate that the basal level of ERα expression was markedly reduced (30–50%) in all of the MCF-7/Cyr61 clones, as compared with the MCF-7/V cells (Figure 3a, left and middle panels). These results indicate that Cyr61 expression is correlated with the loss of ER expression, consistent with our previous finding that Cyr61 expression is closely associated with tumor progression and ER negativity in tumor biopsies (Tsai *et al*., 2000). It has been published recently that Cyr61 expression is associated with diagnosis of advanced diseases of breast cancer (Xie *et al*., 2001), however, the sample number tested was relatively small. Curiously, however, the same report showed that Cyr61 expression in human breast biopsies is correlated with ER positivity (Xie *et al*., 2001; Sampath *et al*., 2001), even though ER expression is known to be an indicator of good prognosis for breast cancer (Brower *et al*., 1999). Therefore, more studies are required to establish the mechanisms of Cyr61 action and its role in breast carcinomas.

We next examined whether Cyr61 promotes loss of ER function by assessing regulation of several well-documented E2-responsive genes. We tested ERα, shown to be downregulated by E2, and progesterone receptor (PgR), Cathepsin D and pS2, all of which have been shown to be upregulated by E2 in MCF-7 cells (Read *et al*., 1989; Nardulli *et al*., 1988; Cavailles *et al*., 1988; Weaver *et al*., 1988). We have previously demonstrated that the loss of PgR regulation by E2 attests for the loss of ER function (Tang *et al*., 1996; Saceda *et al*., 1996). Our studies were then focused on E2 regulation of ERα and PgR expression as previously described (Tang *et al*., 1996). Although the level of ERα expression is lowered, E2 still down-regulates the expression of ERα in MCF-7/Cyr61 cells. This regulation is normally tightly controlled by E2 in the parental MCF-7 cells. Our results demonstrate that in the presence of E2, ERα expression is downregulated about 40–50% in the MCF-7/V2 cells, as compared with the untreated cells (Figure 3a, right panel). In contrast, E2 does not downregulate ERα expression in MCF-7/Cyr61 cells to the same extent as in the MCF-7/V2 cells. E2-induced downregulation of ERα in the MCF-7/Cyr61 cells was only about 10–25% as compared with the untreated cells (Figure 3a, left panel). The diminished effect of E2 on ERα expression is most probably because the basal level of the ERα expression in MCF-7/Cyr61 cells is already markedly reduced (Figure 3a, middle panel), therefore additional treatment with E2 cannot further induce a significant reduction in ERα expression (Figure 3a, right panel).

We then tested whether ER was still a functional receptor for E2 to induce upregulation of PgR gene expression in MCF-7/Cyr61 cells. Interestingly, even though ERα expression was significantly lowered in MCF-7/Cyr61 cells, E2 induced a marked upregulation in PgR mRNA expression. The increase in PgR expression was between 300% and 400%, as compared with the untreated MCF-7/Cyr61 cells (Figure 3b). As expected, E2 upregulated the levels of PgR in the MCF-7/V2 cells by about 200%. Similarly, we observed E2-induced upregulation of other E2-responsive genes, including Cathepsin D and pS2 (data not shown). These data support the notion that ERα is still a functional receptor in MCF-7/Cyr61 cells, although these cells are E2-independent and the level of ERα expression is lower than that in the parental cells. Our combined results demonstrate that Cyr61 is sufficient to confer E2-independence and antiestrogen-resistance. Interestingly, the cells transfected with Cyr61 retained E2-responsiveness and the ERα function. Moreover, we have clearly demonstrated that Cyr61 is capable of downregulating ER expression and promotes an invasive phenotype. This is consistent with our previous data which demonstrates that in breast cancer cells, Cyr61 expression correlates with the loss of ER expression, increased tumorigenicity, and the ability of cells to metastasize *in vivo* (Tsai *et al*., 2000).

To assess the effect of Cyr61 expression *in vivo*, one vector control clone (MCF-7/V2) and four Cyr61 clones (MCF-7/C6, C9, C19, and C20) were inoculated into the mammary fat pads of 4–5 week old ovariectomized athymic nude mice. Two experimental groups (four mice per group) were assessed. One group

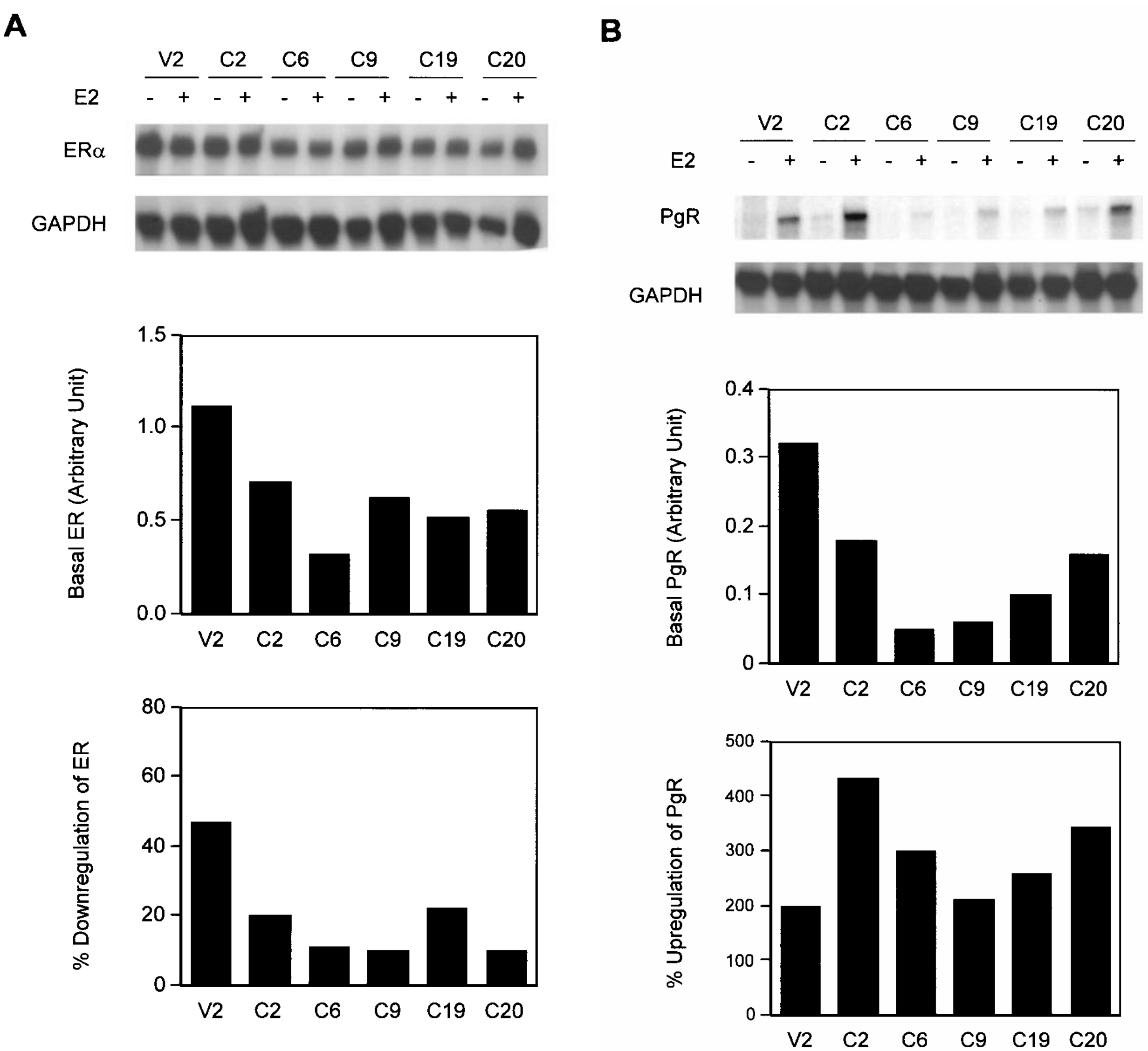

Figure 3 E2-responsive gene expression in MCF-7/Cyr61 clones. Subconfluent MCF-7/V2 and MCF-7/Cyr61 cells were cultured in media containing charcoal-stripped FBS for 4 days and treated in the presence or absence of E2 for 6 h. Total RNA was isolated, and 20 and 30 μg of total RNA were analysed respectively for the expression of (**a**) ERα and (**b**) PgR by the RNase protection assay as previously described (Tang *et al*., 1996). Expression of ERα or PgR was normalized by GAPDH

was studied in the absence of estrogen pellets and the other in the presence of estrogen pellets. Large tumors (ranging between 0.5 cm^3 and 1 cm^3) were spontaneously developed only in those mice inoculated with MCF-7/Cyr61 clones (4/4 for clone C20 and 3/4 for other three clones) just 5 weeks after initial inoculation. Experiments were carried out for 10 weeks during which tumors were growing exponentially with time. These tumors grew independently of hormonal stimulation (Figure 4a). The MCF-7/V2 cells used as a control only developed tumors in the presence of E2 stimulation (Figure 4a). These data demonstrate indisputably that transfection of MCF-7 cells with Cyr61 promotes tumorigenesis *in vivo* in the absence of hormonal stimulation. These findings support our *in vitro* data demonstrating that the MCF-7/Cyr61 cells had a growth advantage in the absence of E2. Significantly, the data from our model system provide novel evidence which resembles many breast cancer clinical cases, showing the progression of the disease from an ER-positive and E2-dependent phenotype to an E2-independent phenotype, yet frequently without the complete loss of ER expression.

The tumors developed by the MCF-7/Cyr61 cells were excised and analysed macroscopically. The majority of the tumors appeared as firm, poorly defined masses. The lesions measured between 1 and 1.5 cm in the greatest dimension. The MCF-7/Cyr61-derived tumors demonstrated fixation to the underlying soft tissues, as well as erosion of the overlying skin. Enlargement of axillary lymph nodes was detected in the same group, whereas no macroscopic metastatic foci to visceral organs were

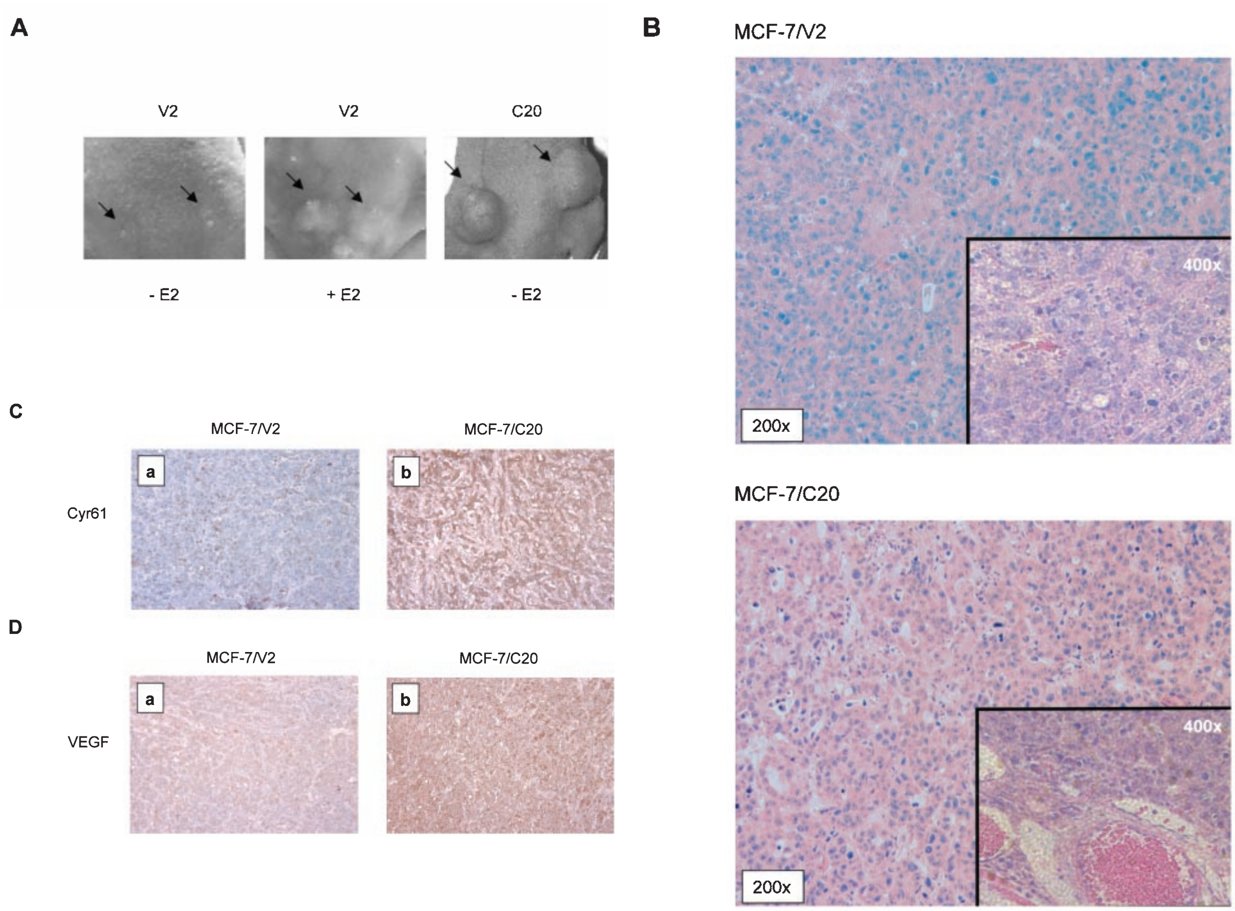

Figure 4 Expression of Cyr61 and VEGF in implanted breast tumor sections from nude mice. (**a**) Photographs of nude mice bearing human breast tumors. Tumors were developed by implanting MCF-7/V2 and MCF-7/Cyr61 cells (2×10^6 cells per site) into the mammary fat pads of 4–5 week old ovariectomized athymic nude mice with or without E2 pellets (25 mg, 90-day slow release) as previously described (Tang *et al*., 1996). (**b**) Hematoxylin and eosin (H&E) staining of human breast tumor sections derived from MCF-7/V2 and MCF-7/Cyr61 clone (clone C20 shown). (**c**) Immunohistochemical analysis of Cyr61 expression in breast tumor sections developed from implanted MCF-7/V2 and MCF-7/Cyr61 cells (clone C20 shown) in nude mice as previously described (Tsai *et al*., 2000). (**d**) Immunohistochemical analysis of VEGF expression in breast tumor sections derived from implanted MCF-7/V2 and MCF-7/Cyr61 cells in nude mice. Microphotographs are shown at $200\times$ magnifications, unless otherwise specified

noticed (Figure 4a). Lymph nodes involvement in the tumors was not observed. In all of the MCF-7/Cyr61 cell groups, the tumors were characterized by histological, tightly cohesive areas of large, pleiomorphic cells with irregular nuclei and numerous mitotic figures. Multinucleated cells were observed. Neovascular formation and areas of necrotic tissues were observed (Figure 4b).

By immunohistochemical staining using an anti-Cyr61 antibody, we observed very high levels of Cyr61 expression in the tumors developed from MCF-7/Cyr61 cells. On the contrary, the levels of Cyr61 expression in tumors developed with the control MCF-7/V2 cells in the presence of E2 were very low or undetectable (Figure 4c). These data demonstrate that the expression of Cyr61 is maintained *in vivo*, and that the phenotypic changes are mediated most probably through the Cyr61 protein. The tumors developed from the Cyr61-expressing cells were evidently vascularized (Figure 4a,b). We therefore tested the expression of another angiogenic factor apart from Cyr61, the vascular endothelial growth factor (VEGF), a growth factor to stimulate neovascularization. Using immunohistochemical staining with an anti-VEGF antibody, a marked increase in VEGF expression was observed in the tumor sections derived from the MCF-7/Cyr61 cells, but not in sections derived from MCF-7/V2 tumors formed in the presence of E2 (Figure 4d). The detection of the VEGF expression by immunohistochemical staining is specific, because no background staining was observed when using the control peptide-neutralized anti-VEGF antibody (data not shown). Moreover, our results confirm a recent report showing that Cyr61 regulates VEGF expression in primary skin fibroblasts (Chen *et al*., 2001).

Overall, the role of Cyr61 in breast cancer is still under investigation and further studies are necessary to determine the mechanism by which Cyr61 promotes breast cancer progression. We have clearly established that Cyr61 promotes tumor growth of breast cancer cells, in accordance with earlier observations, showing that a gastric adenocarcinoma cell line RF1 becomes tumorigenic when Cyr61 is introduced in those cells (Babic *et al*., 1998). While this work and manuscript were in progress, another study described the ability of Cyr61 to induce tumorigenicity of breast cancer and breast normal cells (Xie *et al*., 2001). However, the study addresses neither the ER expression/function, nor the extensive biological characterization as we have presented here.

In this study, we demonstrate that expression of Cyr61 leads to E2-independence and antiestrogen resistance in MCF-7 cells for both anchorage-dependent and -independent growth. In the Matrigel matrix, Cyr61 promotes outgrowth of the MCF-7 cells in an E2-independent manner. Apparently, Cyr61 down-regulates the expression of ERα yet does not disrupt its function. Thus, Cyr61-expressing cells are still E2-responsive. We also demonstrate that overexpression of Cyr61 induces tumor formation in immunodeficient mice and promotes the expression of VEGF, an important regulator of neovascularization. Our current results further imply that Cyr61 is a downstream effector of HRG, because Cyr61 can bypass the effect of HRG and induce similar phenotypic changes of breast cancer cells as those promoted by HRG.

It has been shown that Cyr61 is an angiogenic ligand for $\alpha v\beta 3$ (Babic *et al*., 1998, 1999). We have previously reported that a functional blocking antibody against $\alpha v\beta 3$ is capable of blocking HRG induction of the aggressive phenotypes of the breast cancer cells (Tsai *et al*., 2000). We have proposed that Cyr61 mediates tumor growth and angiogenesis of breast cancer cells in either an autocrine or paracrine manner through its binding to the $\alpha v\beta 3$ integrin receptor. This receptor is often expressed in endothelial as well as epithelial cells. Integrin receptors have been shown to mediate cellular adhesion to the extracellular matrix, which is known to exert profound control over the cells. Although Cyr61 binds to several integrins $\alpha v\beta 3$, $\alpha IIb\beta 3$ and $\alpha 6\beta 1$, so far only the $\alpha v\beta 3$ integrin has been shown to play a major role in breast cancer tumor vascularization and progression (Meyer *et al*., 1998). More importantly, it has been recently demonstrated that overexpression of $\alpha v\beta 3$ is a marker for poor prognosis in breast cancer (Gasparini *et al*., 1998). We can then postulate that binding of Cyr61 to the angiogenic integrin receptor $\alpha v\beta 3$ should provide new insights into the possible mechanisms by which Cyr61 promotes breast tumorigenesis and cancer progression. We are currently investigating this avenue that will bring to light further molecular mechanisms of Cyr61 action.

Acknowledgments

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